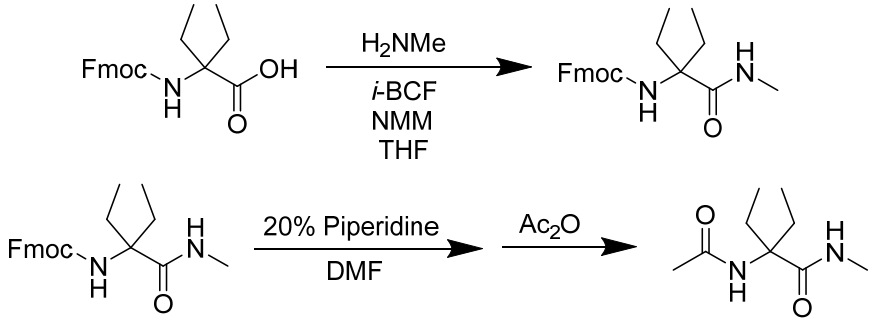
Supplementary Note 2 | Synthesis of diethylglycine derivatives and peptides

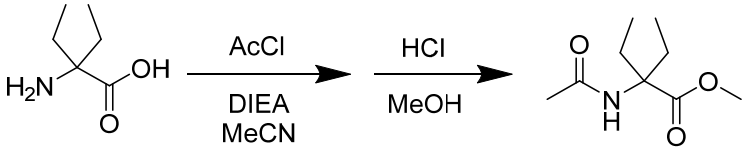
Synthesis of diethylglycine derivatives (General methods). Commercial chemicals were of reagent grade or better, and were used without further purification. Amino acid starting materials and peptide resins were obtained from Chem-Impex International. All other reagents were obtained from Sigma–Aldrich. Anhydrous THF and DMF were obtained from CYCLE-TAINER solvent-delivery systems from J. T. Baker (Phillipsburg, NJ). Reactions were monitored by thin-layer chromatography with visualization by UV light or staining with $KMnO_4$ or ninhydrin. Chromatography was performed with columns of silica gel 60, 230–400 mesh (Silicycle, Québec City, Canada). The removal of solvents and other volatile materials "under reduced pressure" refers to the use of a rotary evaporator at water-aspirator pressure (<20 torr) and a water bath of <45 °C.

Nuclear magnetic resonance (NMR) spectra for compound characterization were acquired at ambient temperature with an Avance III 500 MHz spectrometer (^{13}C, 126 MHz). ^{13}C spectra were proton-decoupled. Mass spectrometry was performed with a Micromass LCT (electrospray ionization, ESI) instrument from Waters (Milford, MA) in the Mass Spectrometry Facility of the Department of Chemistry at the University of Wisconsin–Madison.

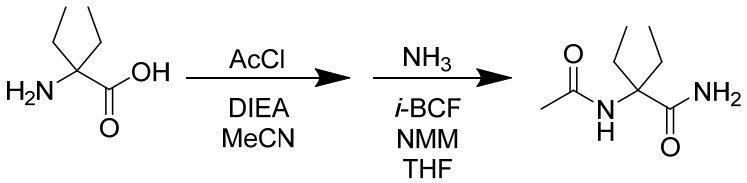


Synthesis of *N*-acetyldiethylglycine methyl amide (AcDegNHMe). Fmoc-Diethylglycine (1.0 g, 2.8 mmol) was dissolved in 50 mL of anhydrous THF containing NMM (0.62 mL, 5.6 mmol). The mixture was cooled to –20 °C and isobutyl chloroformate (0.37 mL, 2.8 mmol) was added dropwise. After 10 min, 3 mL of 2 M methylamine in THF was added, and the reaction mixture was stirred overnight, allowing warmth to room temperature. The solution was filtered, and solvent was removed under reduced pressure. The residue was dissolved in EtOAc and washed with 1 M aqueous KH_2PO_4, saturated aqueous $NaHCO_3$, and brine. The organic portion was dried over anhydrous $NaSO_4$(s), and solvent was removed under reduced pressure. The residue was purified by chromatography on silica gel using an eluent of 2% v/v MeOH in DCM containing 1% v/v TEA, which afforded FmocDegNHMe as a white powder. The solid was dissolved in 20% v/v piperidine in DMF, and this solution was stirred for 1 h, after which the solvent was removed under reduced pressure. The residue was then dissolved in acetic anhydride and stirred overnight. After removing the solvent under reduced pressure, chromatography on silica gel using an eluent of 3% v/v MeOH in DCM afforded AcDegNHMe as a crystalline solid. ^1H NMR (500 MHz, $CDCl_3$, δ): 6.91 (s, 1H), 6.33 (s, 1H), 2.88 (d, J = 4.5 Hz, 3H), 2.59 (dq, J = 14.0, 7.4 Hz, 2H), 2.03 (s, 3H), 1.55 (dq, J = 14.0, 7.4 Hz, 2H), 0.76 (t, J =

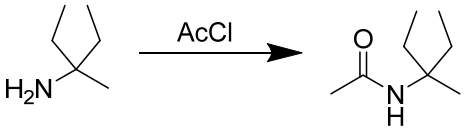
7.4 Hz, 6H); ^{13}C NMR (126 MHz, $CDCl_3$, δ): 173.7, 169.2, 65.3, 28.8, 26.7, 24.3, 8.3; ESI–MS: $[M + H]^+$ calculated 187.1442, found 187.1439.



Synthesis of *N*-acetyldiethylglycine methyl ester (AcDegOMe). Diethylglycine (1.0 g, 7.6 mmol) was suspended in acetonitrile with TEA (2.1 mL, 15 mmol). Acetyl chloride (0.54 mL, 7.6 mmol) was added dropwise, and the reaction mixture was stirred at room temperature overnight. The solid was collected by filtration and purified by chromatography on silica gel in 12% v/v MeOH in DCM with 1% v/v TFA to remove residual starting material. The resulting solid was dissolved in MeOH, acidified with concentrated HCl, and heated at reflux for 1 h. After removing the solvent under reduced pressure, the residue was purified by chromatography on silica gel using an eluent of 4% v/v MeOH in DCM to afford AcDegOMe as a crystalline solid. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 6.36 (s, 1H), 3.78 (s, 3H), 2.48 (dq, J = 14.0, 7.4 Hz, 2H), 2.03 (s, 3H), 1.77 (dq, J = 14.0, 7.4 Hz, 2H), 0.74 (t, J = 7.4 Hz, 6H); ^{13}C NMR (126 MHz, $CDCl_3$, δ): 174.8, 168.9, 66.3, 52.8, 27.9, 24.2, 8.6; ESI–MS: $[M + H]^+$ calculated 188.1282, found 188.1276.



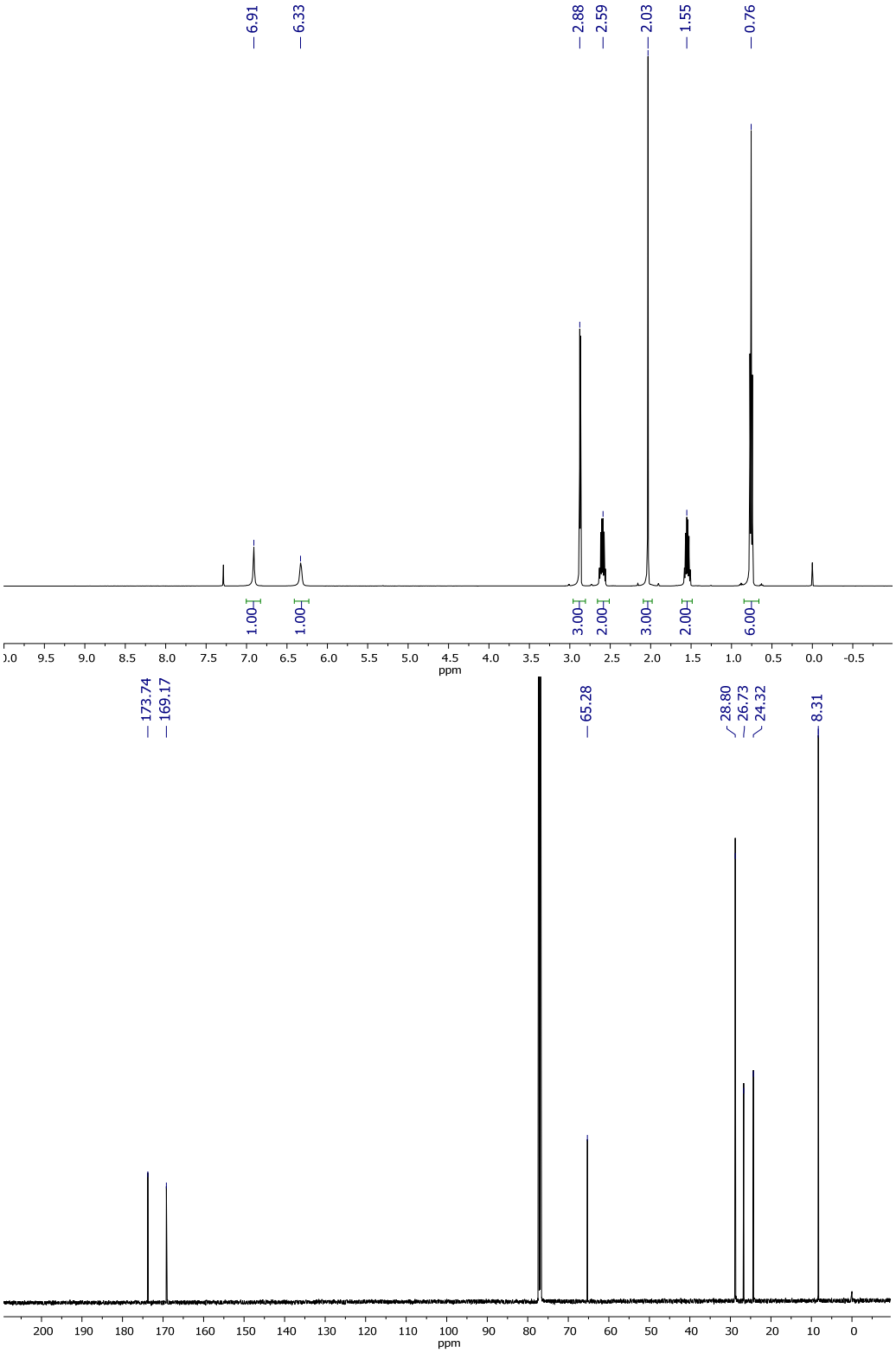
Synthesis of *N*-acetyldiethylglycine amide ($AcDegNH_2$). Diethylglycine (1.0 g, 7.6 mmol) was suspended in acetonitrile with TEA (2.1 mL, 15 mmol). Acetyl chloride (0.54 mL, 7.6 mmol) was added dropwise, and the reaction mixture was stirred at room temperature overnight. The solid was collected by filtration and purified by chromatography of silica gel using an eluent of 12% v/v MeOH in DCM containing 1% v/v TFA. The resulting solid (0.15 g, 0.9 mmol) was dissolved in anhydrous THF containing NMM (0.19 mL, 1.8 mmol), and this solution was cooled to –20 °C. Isobutyl chloroformate (0.11 mL, 0.9 mmol) was added dropwise. After 10 min, 3 mL of 7 N ammonia in MeOH was added, and the mixture was stirred overnight, allowing warmth to room temperature. The solution was filtered, and solvent removed under reduced pressure. The residue was purified by reverse-phase HPLC (A = 0.1% v/v TFA in H_2O; B = 0.1% v/v TFA in MeCN) on a preparative C18 column using a linear gradient of B (5–95% v/v) over 30 min. Lyophilization yielded $AcDegNH_2$ as a white powder. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 6.77 (s, 1H), 6.05 (s, 1H), 5.79 (s, 1H), 2.60 (dq, J = 14.0, 7.4 Hz, 2H), 2.02 (s, 3H), 1.56 (dq, J = 14.0, 7.4 Hz, 2H), 0.80 (t, J = 7.4 Hz, 6H); ^{13}C NMR (126 MHz, $CDCl_3$, δ): 175.7, 169.5, 65.4, 28.8, 24.3, 8.3; ESI–MS: $[M + H]^+$ calculated 173.1285, found 173.1286.



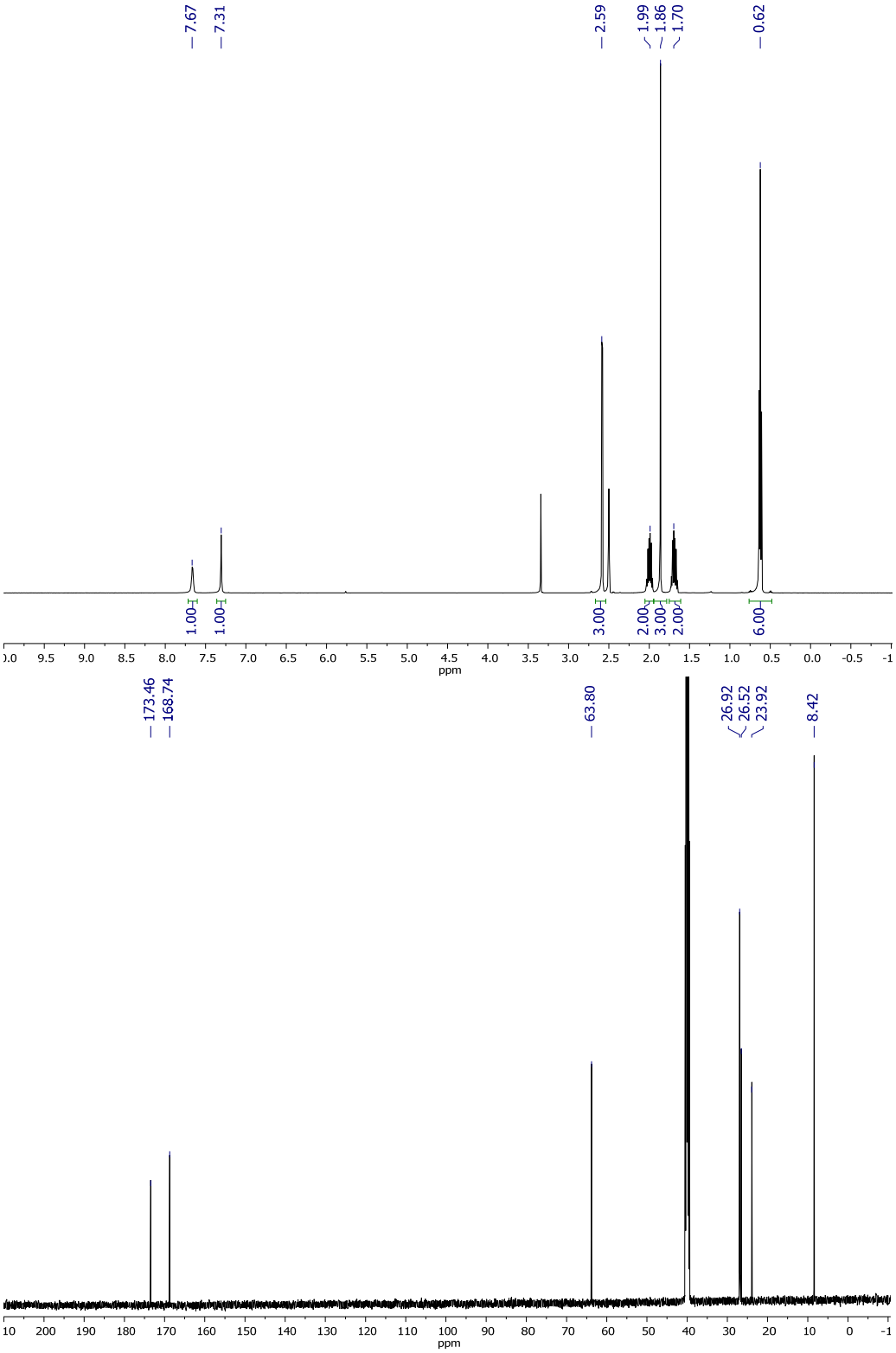
Synthesis of 3-acetamido-3-methylpentane. 3-Amino-3-methylpentane (0.10 g, 1.0 mmol) was dissolved in acetyl chloride, and the reaction mixture was stirred for 1 h. The solution was diluted with DCM and washed with water, 1 M HCl, and saturated aqueous $NaHCO_3$. The organic portion was dried over anhydrous $NaSO_4$(s). Removal of solvent under reduced pressure afforded 3-acetamido-3-methylpentane as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 5.01 (s, 1H), 1.93 (s, 3H), 1.77 (dq, J = 14.0, 7.4 Hz, 2H), 1.62 (dq, J = 14.0, 7.4 Hz, 2H), 1.20 (s, 3H), 0.82 (t, J = 7.4 Hz, 6H); ^{13}C NMR (126 MHz, $CDCl_3$, δ): 169.4, 56.7, 30.3, 24.5, 23.3, 8.0; ESI–MS: $[M + H]^+$ calculated 144.1383, found 144.1385.

Synthesis of TrpZip peptides. Peptide synthesis was performed with a Prelude automated synthesizer from Protein Technologies (Tucson, AZ) in the University of Wisconsin–Madison Biotechnology Center. Peptide purification was accomplished with an LC-20 HPLC instrument from Shimadzu (Kyoto, Japan). Peptide characterization was performed with an LCMS 2020 instrument from Shimadzu.

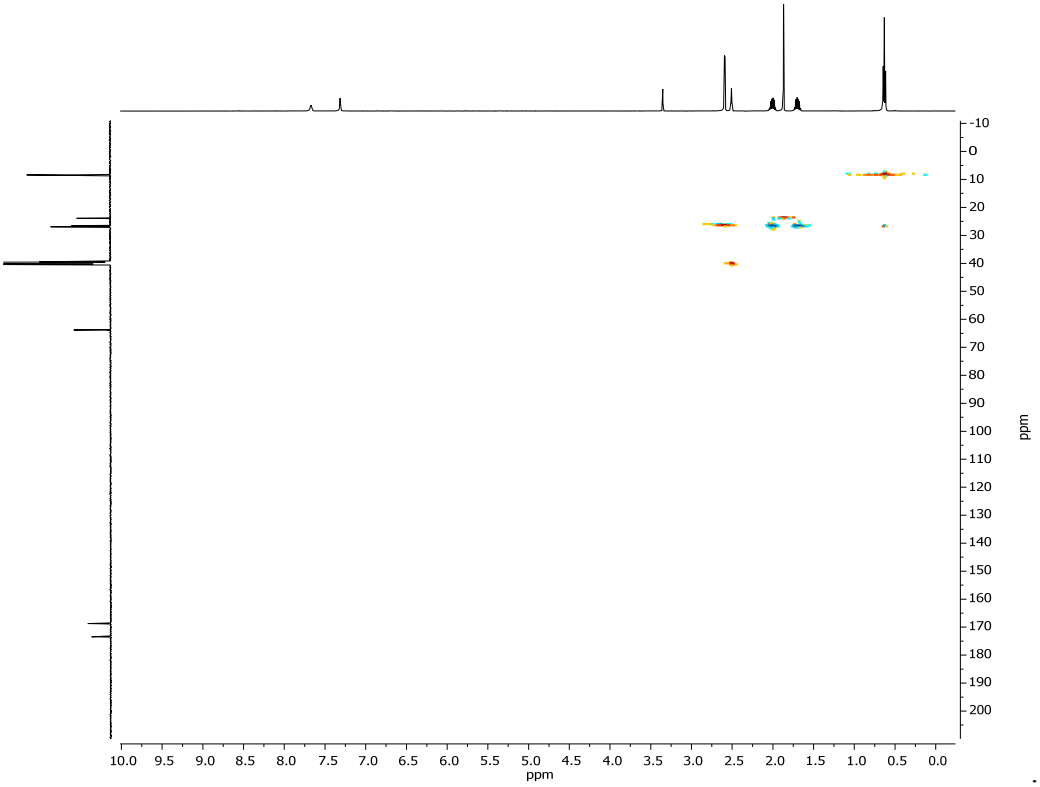
α-Hydroxy acids were prepared as described previously[1]. Ester linkages were forged in solution by activation with DIC/DMAP to yield dimers suitable for solid-phase synthesis. Solid-phase peptide synthesis was conducted with Fmoc chemistry on TentaGel S RAM resin, except for TrpZip2-F, which was synthesized on TentaGel HMBA resin. Amide-bond formation was accomplished by activation of 4 equiv of amino acid with 4 equiv of HCTU in the presence of 8 equiv of NMM for 1 h. N-Terminal acetylation was achieved by treatment with acetic anhydride or trifluoroacetic anhydride, as required. Release and global deprotection were performed by treatment of resin with a solution of 90% v/v TFA, 5% v/v phenol, 2.5% v/v TIPS, and 2.5% v/v H_2O for 3 h. For TrpZip2-F, release and global deprotection were performed according to procedures reported previously[2]. Following precipitation in anhydrous diethyl ether, peptides were isolated by centrifugation and dissolved in 25% v/v MeCN in H_2O. Peptides were purified by reversed-phase HPLC on a preparative NucleoSil C18 column from Macherey–Nagel (Bethlehem, PA) using a linear gradient of 25–50% v/v B over 45 min (A: 0.1% v/v TFA in H_2O; B: 0.1% v/v TFA in MeCN) followed by lyophilization to yield white powders. Purified peptides were analyzed by LC–MS using an analytical Supelco Discovery BIO Wide Pore C5-5 column from Sigma–Aldrich with a linear gradient of 5–95% v/v B over 15 min (A: 0.1% v/v formic acid in H_2O; B: 0.1% v/v formic acid in MeCN).

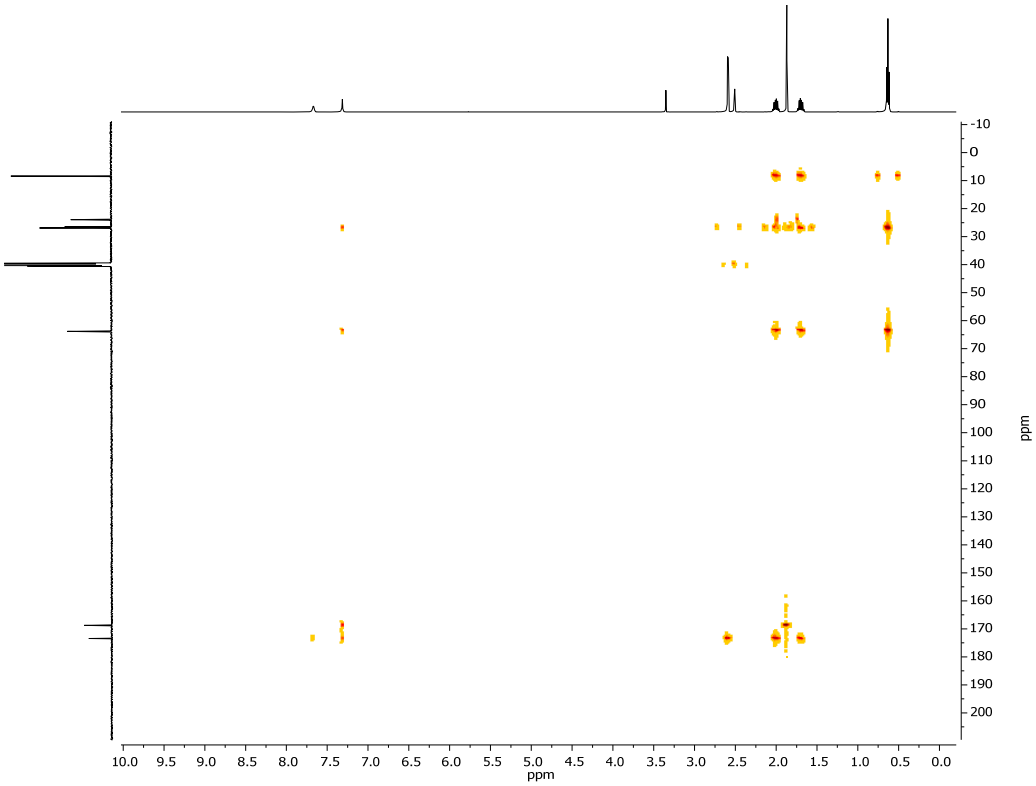


^{1}H and ^{13}C NMR spectra of AcDegNHMe in $CDCl_3$.

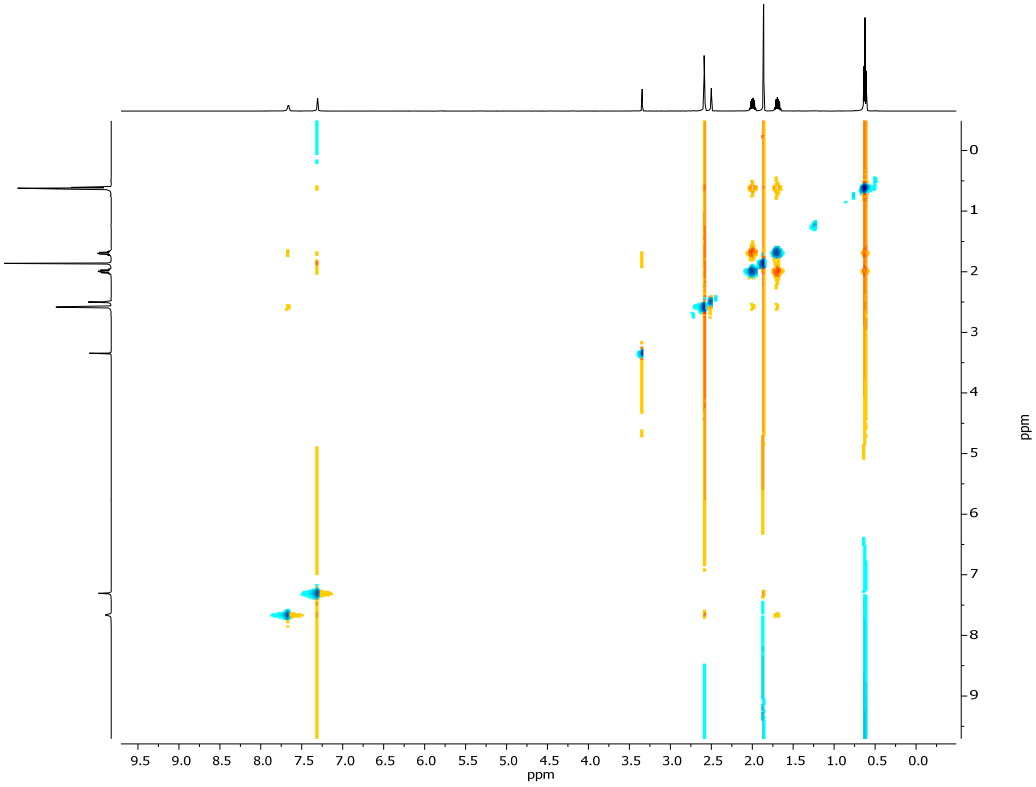


^{1}H and ^{13}C NMR spectra of AcDegNHMe in DMSO-d_6.

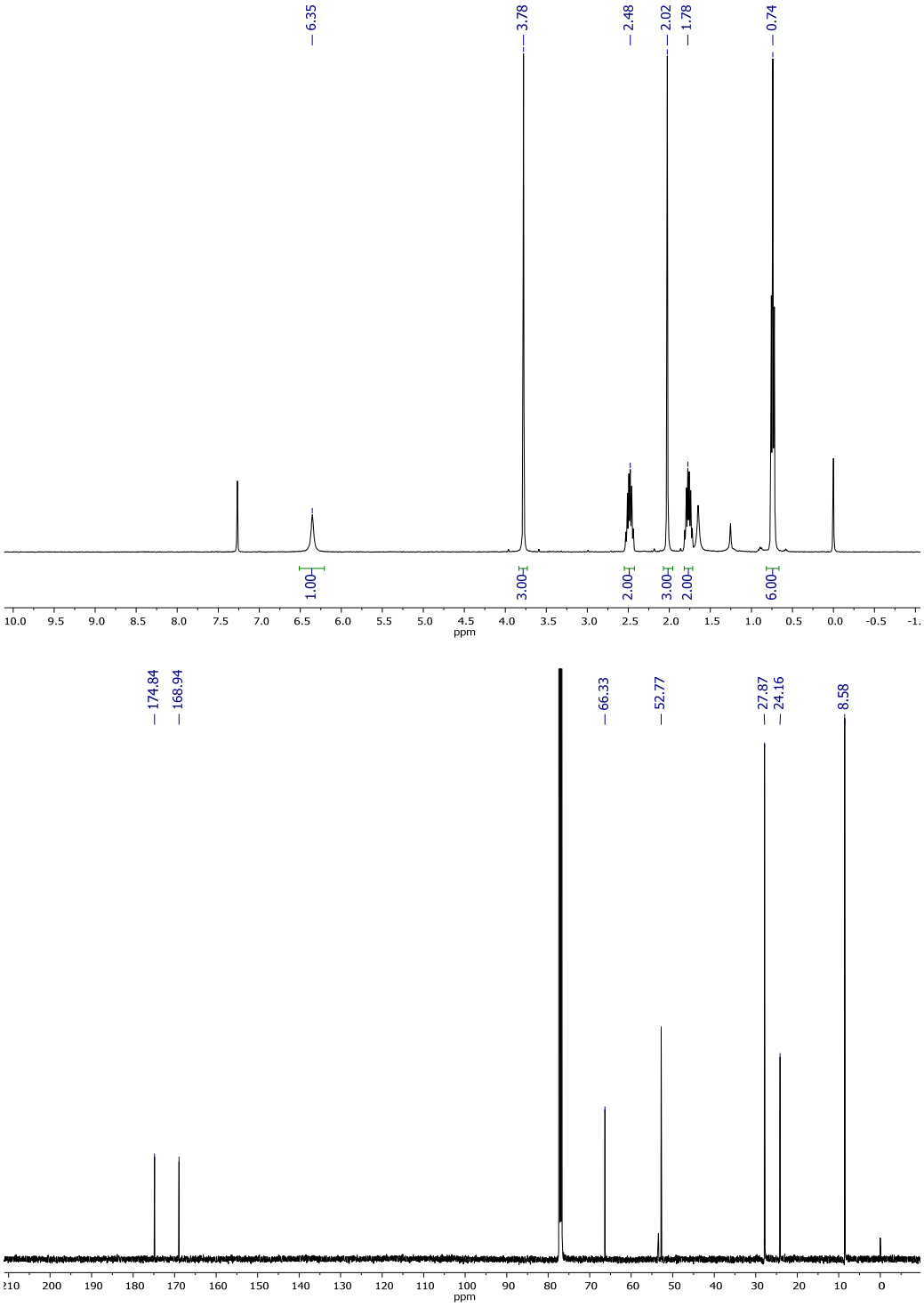




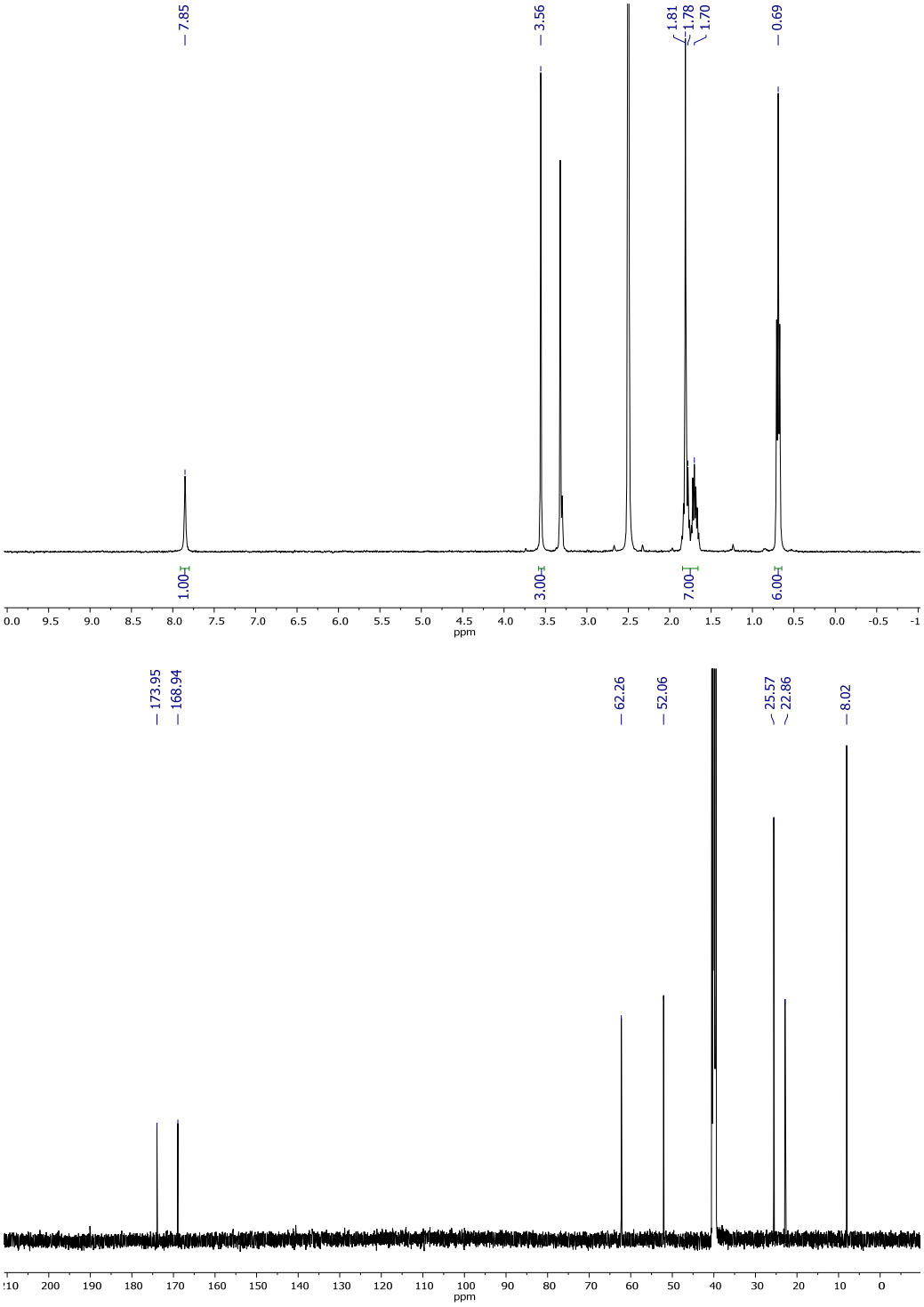
^{1}H–^{13}C HSQC and HMBC spectra of AcDegNHMe in DMSO-d_6.



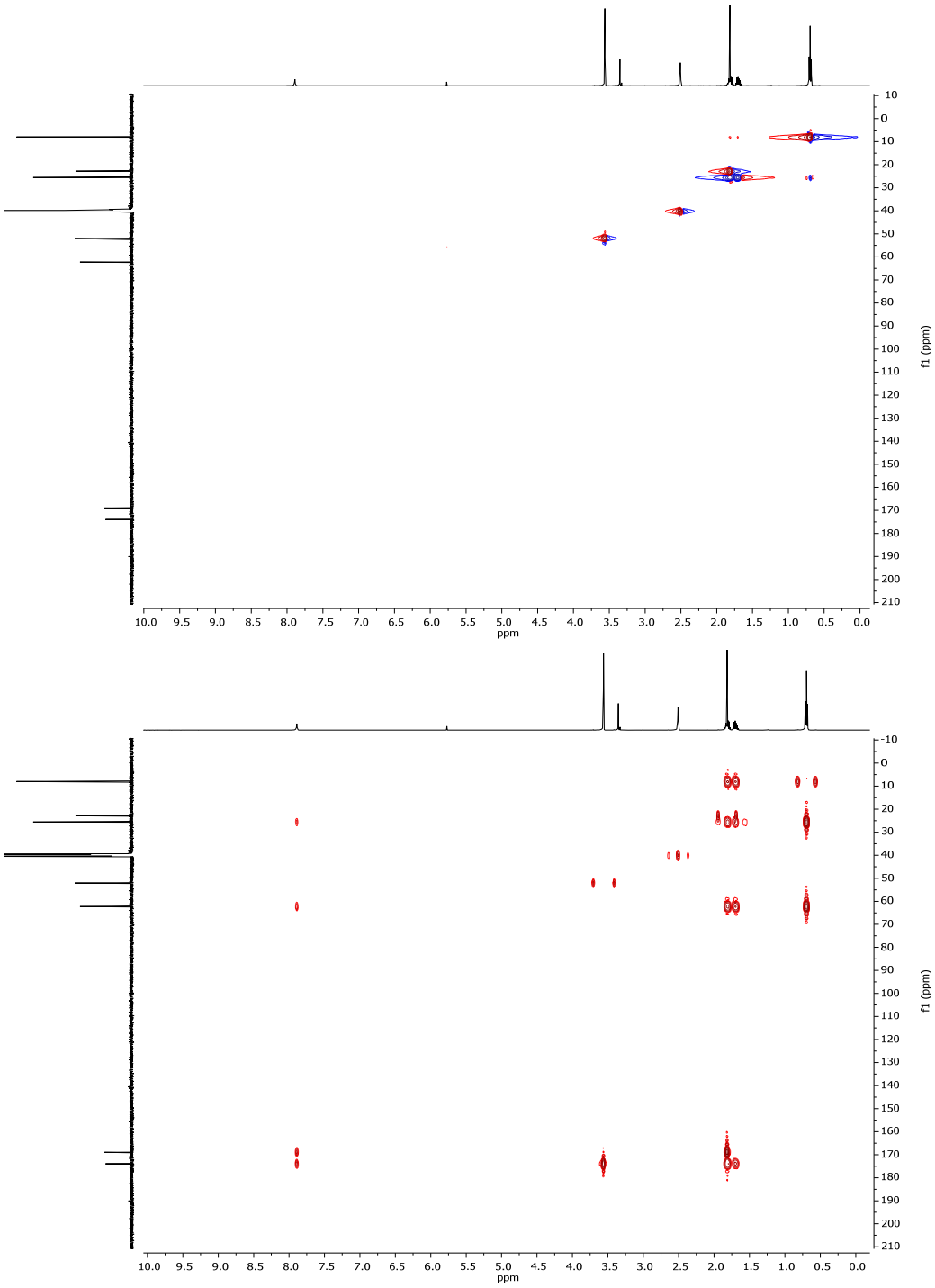
^{1}H–^{1}H NOESY spectrum of AcDegNHMe in DMSO-d_6.



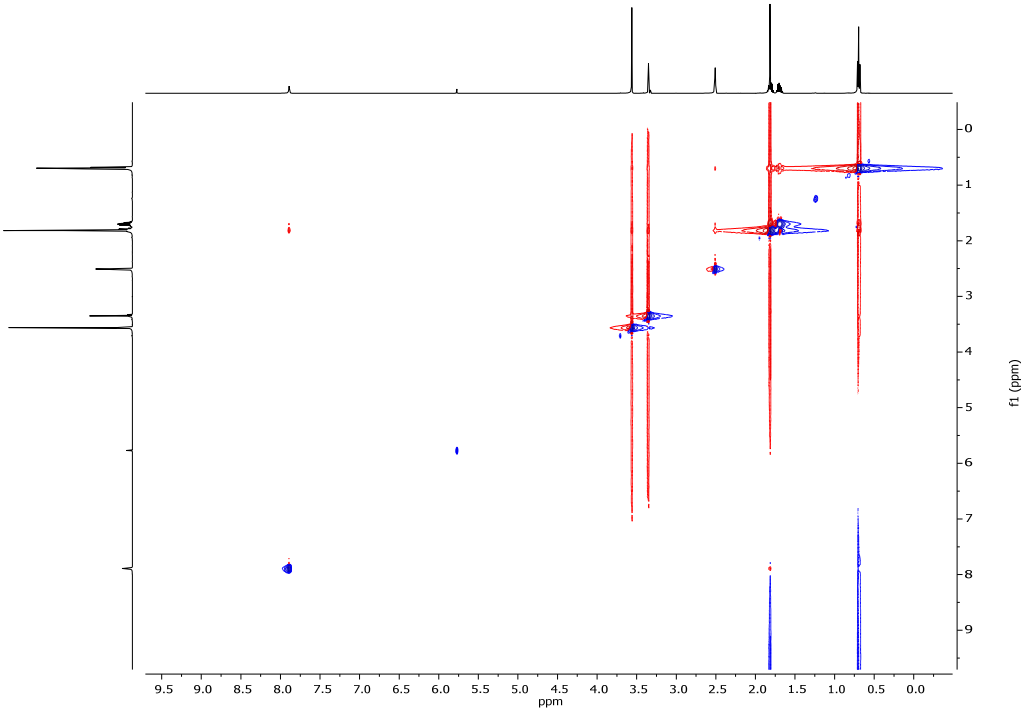
^{1}H and ^{13}C NMR spectra of AcDegOMe in $CDCl_3$.



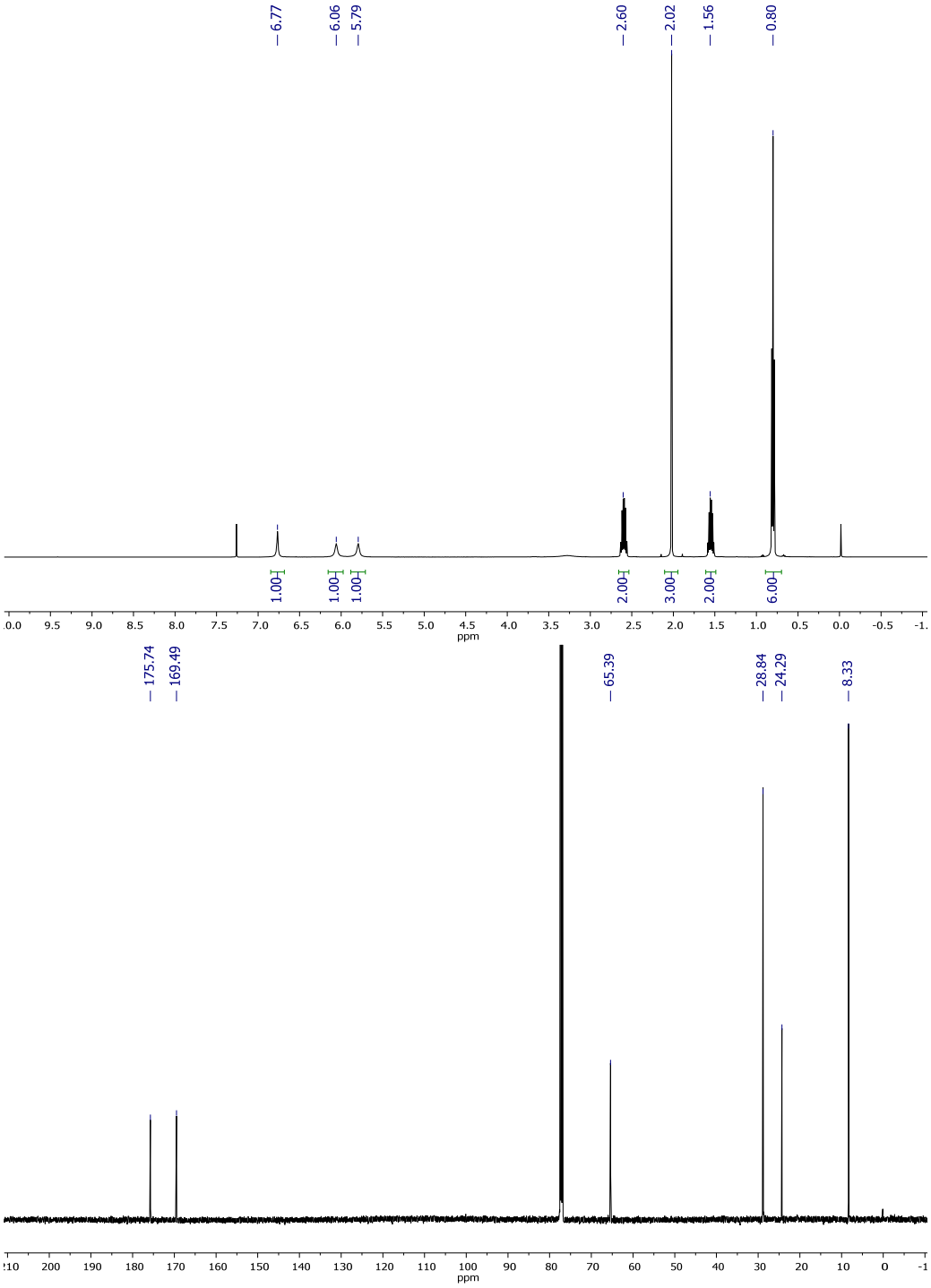
^{1}H and ^{13}C NMR spectra of AcDegOMe in DMSO-d_6.



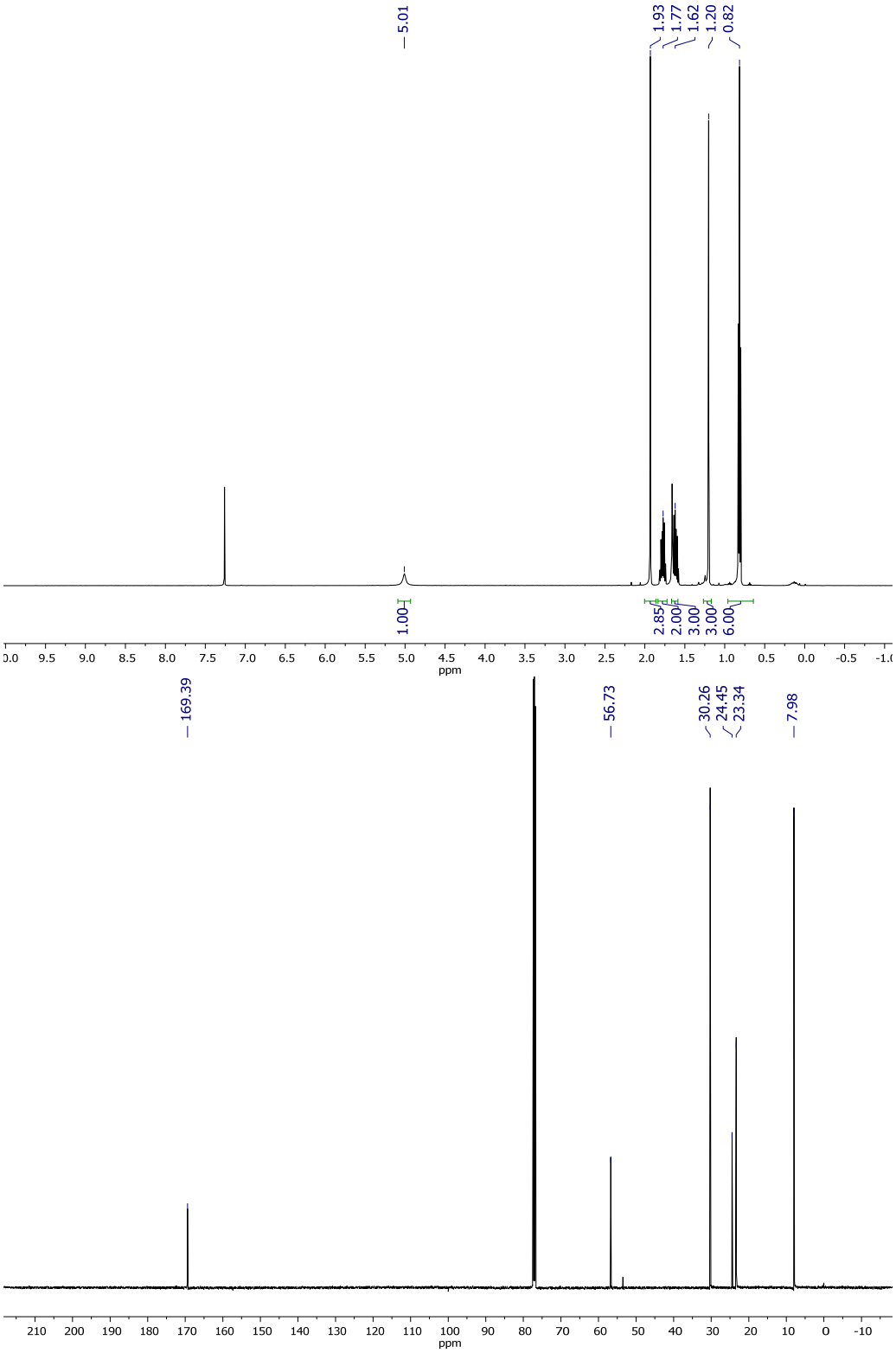
^{1}H–^{13}C HSQC and HMBC spectra of AcDegOMe in DMSO-d_6.



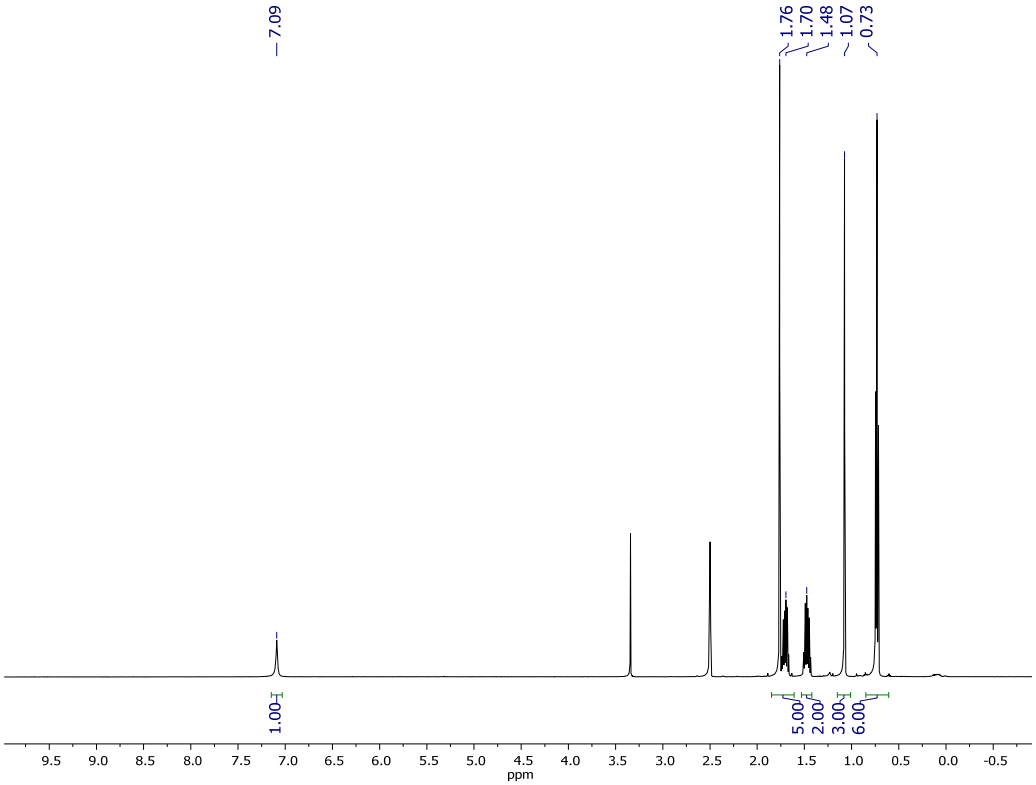
^{1}H–^{1}H NOESY spectrum of AcDegOMe in DMSO-d_6.

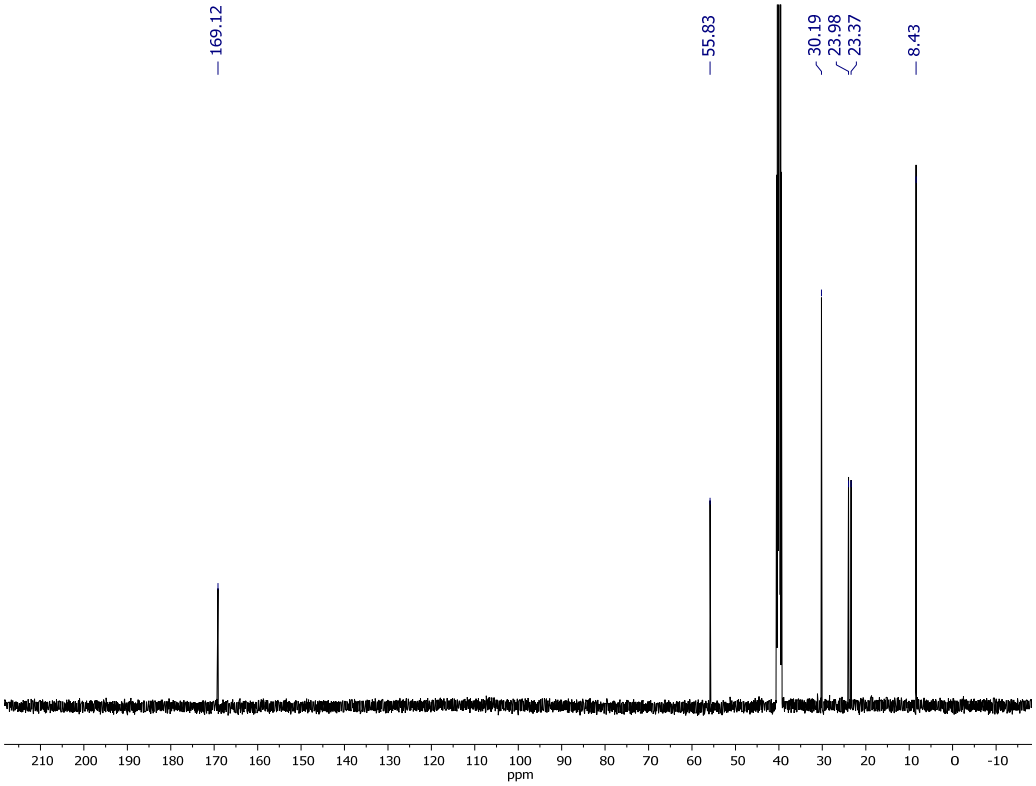


^{1}H and ^{13}C NMR spectra of $AcDegNH_2$ in $CDCl_3$.

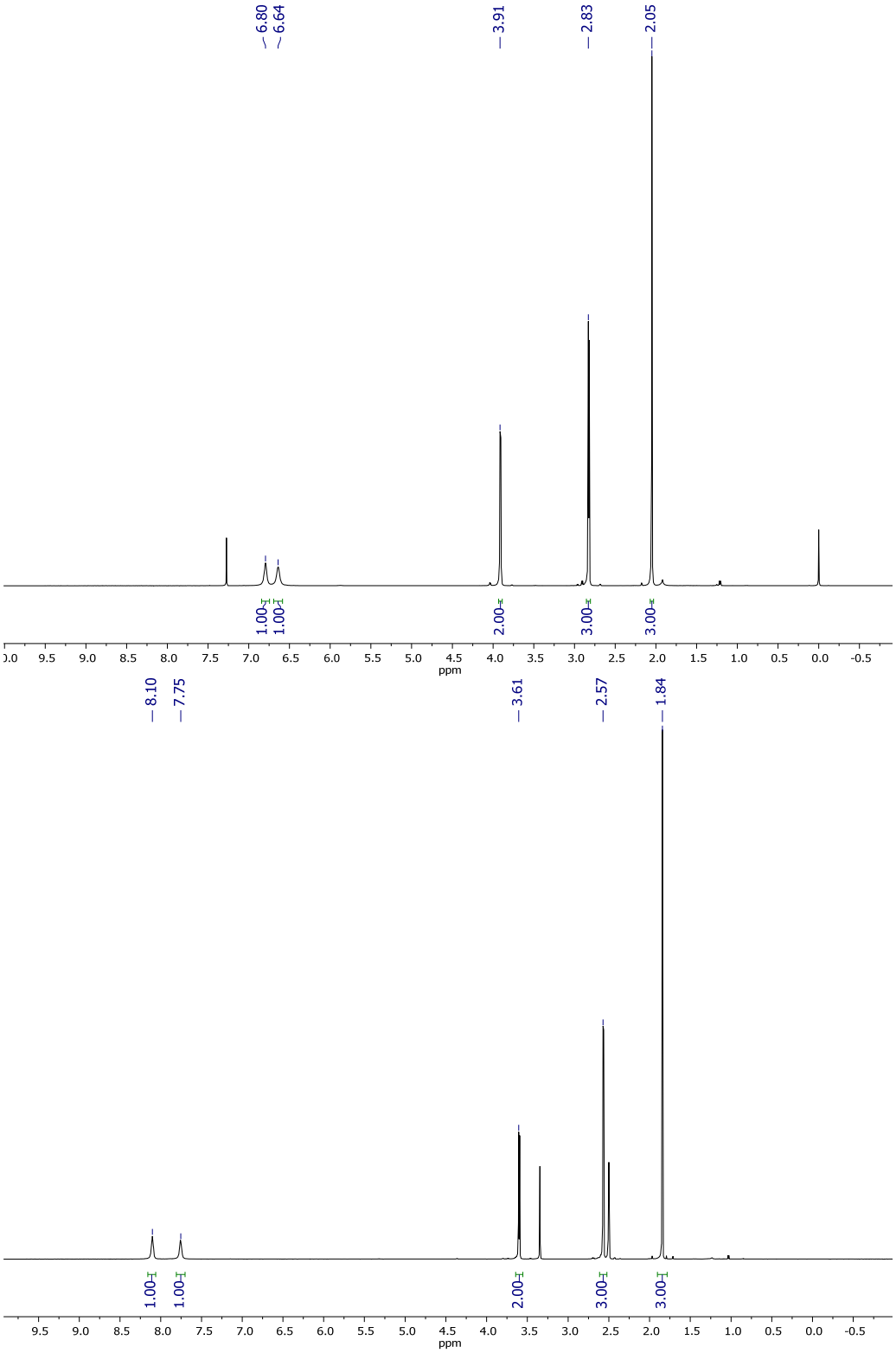


^{1}H and ^{13}C NMR spectra of 3-acetamido-3-methylpentane in $CDCl_3$.

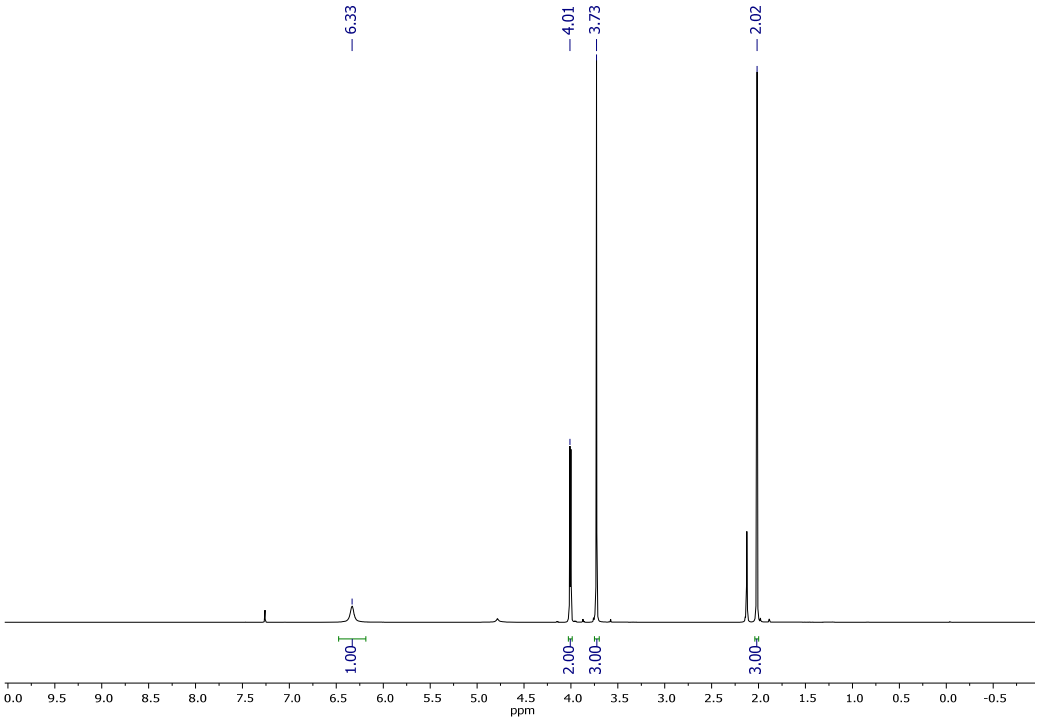


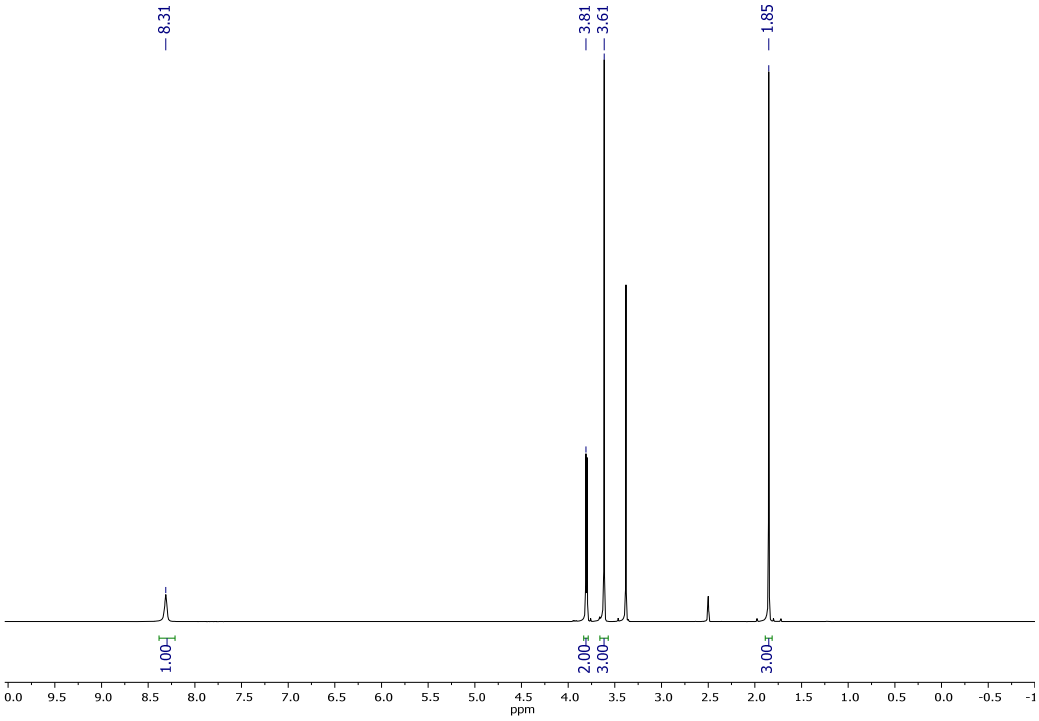


^{1}H and ^{13}C NMR spectra of 3-acetamido-3-methylpentane in DMSO-d_6.

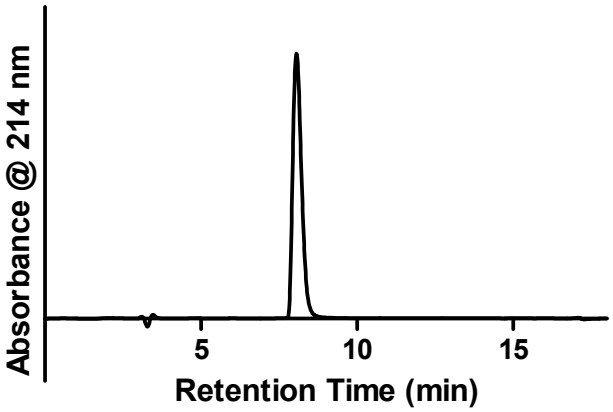


^{1}H NMR spectra of AcGlyNHMe in $CDCl_3$ and DMSO-d_6.

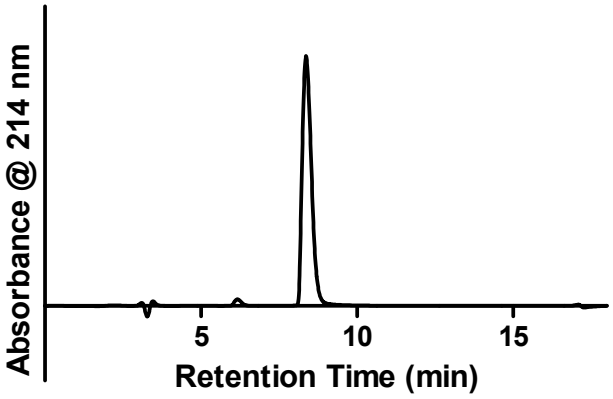




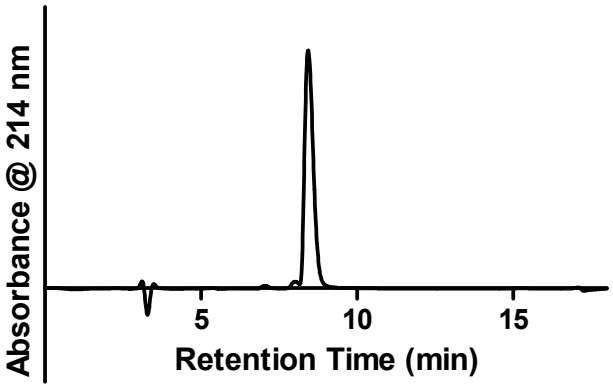
^{1}H NMR spectra of AcGlyOMe in $CDCl_3$ and DMSO-d_6.



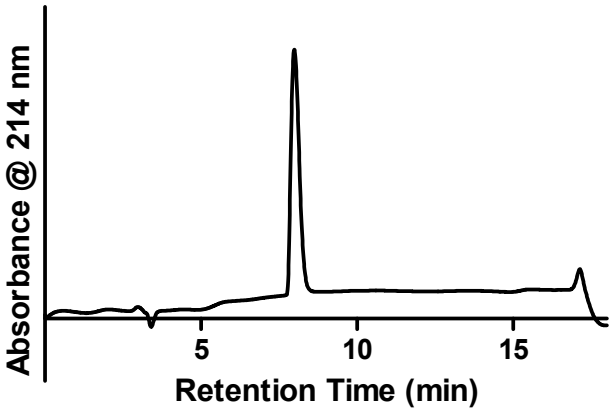
Analytical LC–MS trace of purified TrpZip2-A. Calculated monoisotopic exact mass for $[M+H]^+$ ($C_{80}H_{105}N_{20}O_{19}$): 1649.78; found: 1649.55.



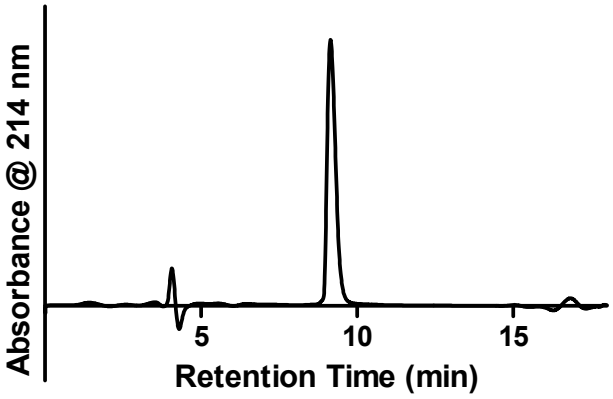
Analytical LC–MS trace of purified TrpZip2-B. Calculated monoisotopic exact mass for $[M+H]^+$ ($C_{80}H_{105}N_{19}O_{20}$): 1650.76; found: 1650.55.



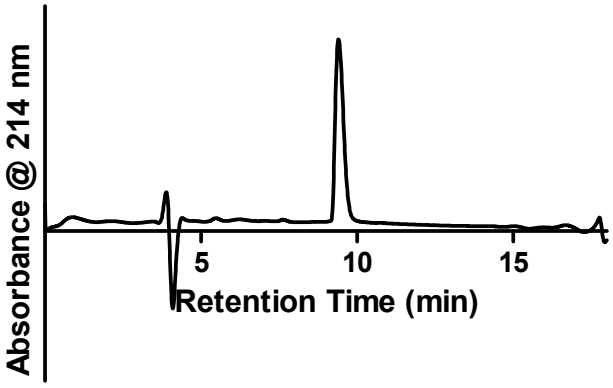
Analytical LC–MS trace of purified TrpZip2-C. Calculated monoisotopic exact mass for $[M+H]^+$ ($C_{80}H_{105}N_{19}O_{20}$): 1650.76; found: 1650.50.



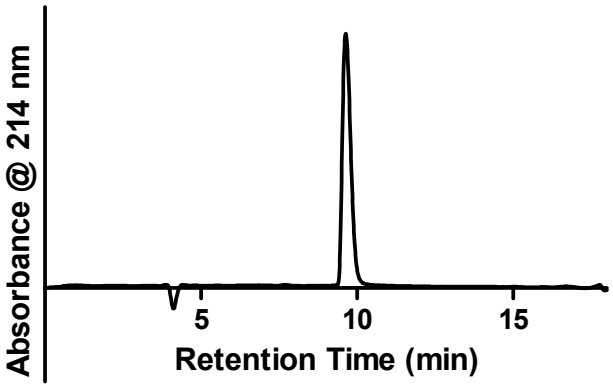
Analytical LC–MS trace of purified TrpZip2-D. Calculated monoisotopic exact mass for $[M+H]^+$ ($C_{80}H_{105}N_{18}O_{21}$): 1651.75; found: 1651.50.



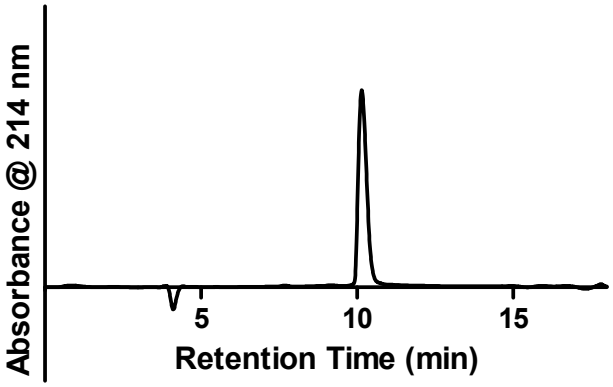
Analytical LC–MS trace of purified TrpZip2-E. Calculated monoisotopic exact mass for $[M+H]^+$ ($C_{77}H_{100}N_{19}O_{17}$): 1562.75; found: 1562.55.



Analytical LC–MS trace of purified TrpZip2-F. Calculated monoisotopic exact mass for $[M+H]^+$ ($C_{78}H_{101}N_{18}O_{18}$): 1577.75; found: 1577.60.



Analytical LC–MS trace of purified TrpZip2-G. Calculated monoisotopic exact mass for $[M+H]^+$ ($C_{74}H_{93}N_{18}O_{18}$): 1521.68; found: 1521.60.



Analytical LC–MS trace of purified TrpZip2-H. Calculated monoisotopic exact mass for $[M+H]^+$ ($C_{74}H_{90}N_{18}O_{18}F_3$): 1575.66; found: 1575.60.

References

1. Deechongkit, S., You, S.-L. & Kelly, J.W. Synthesis of all nineteen appropriately protected chiral α-hydroxy acid equivalent of the α-amino acids for Boc solid-phase depsi-peptide synthesis. *Org. Lett.* **6**, 497-500 (2004).
2. Abd-Elgaliel, W.R., Gallazzi, F. & Lever, S.Z. Total solid-phase synthesis of bombesin analogs with different functional groups at the C-terminus. *J. Peptide Sci.* **13**, 487-492 (2007).